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Functional and molecular biological evidence for a possible β_3-adrenoceptor in the human detrusor muscle

*,[1]Yasuhiko Igawa, [2]Yoshinobu Yamazaki, [2]Hiroo Takeda, [2]Kohichi Hayakawa, [2]Masuo Akahane, [2]Yukiyoshi Ajisawa, [1]Takehisa Yoneyama, [1]Osamu Nishizawa & [3]Karl-Erik Andersson

[1]Department of Urology, Shinshu University School of Medicine, 3-1-1, Asahi, Matsumoto 390-8621, Japan; [2]Division of Discovery Research, Kissei Pharmaceutical Co., Ltd., Hotaka, Nagano 399-8304, Japan; [3]Department of Clinical Pharmacology, Lund University Hospital, S-221 85 Lund, Sweden

1 The possible existence of a β_3-adrenergic receptor (β_3-AR) in the human detrusor muscle was investigated by *in vitro* functional studies and analysis of mRNA expression.

2 Isoprenaline, noradrenaline and adrenaline each produced a concentration-dependent relaxation of the human detrusor. The rank order for their relaxing potencies was isoprenaline (pD_2 6.37 ± 0.07) $\geqslant$ noradrenaline (pD_2 6.07 ± 0.12) $\geqslant$ adrenaline (pD_2 5.88 ± 0.11).

3 Neither dobutamine (β_1- and β_2-AR agonist) nor procaterol (β_2-AR agonist) produced any significant relaxation at concentrations up to 10^{-5} M. BRL37344A, CL316243 and CGP-12177A (β_3-AR agonists), relaxed the preparations significantly at concentrations higher than 10^{-6} M. The pD_2 values for BRL37344A, CL316243 and CGP-12177A were 6.42 ± 0.25, 5.53 ± 0.09 and 5.74 ± 0.14, respectively.

4 CGP-20712A (10^{-7}–10^{-5} M), a β_1-AR antagonist, did not affect the isoprenaline-induced relaxation. On the other hand, ICI-118,551, a β_2-AR antagonist, produced a rightward parallel shift of the concentration-relaxation curve for isoprenaline only at the highest concentration used (10^{-5} M) and its pK_B value was 5.71 ± 0.19. Moreover, SR58894A (10^{-7}–10^{-5} M), a β_3-AR antagonist, caused a rightward shift of the concentration-relaxation curve for isoprenaline in a concentration-dependent manner. The pA_2 value and slope obtained from Schild plots were 6.24 ± 0.20 and 0.68 ± 0.31.

5 The β_1-, β_2- and β_3-AR mRNAs were all positively expressed in detrusor smooth muscle preparations in a reverse transcription polymerase chain reaction assay.

6 In conclusion, the present results provide the first evidence for the existence of the β_3-AR subtype in the human detrusor. They also suggest that the relaxation induced by adrenergic stimulation of the human detrusor is mediated mainly through β_3-AR activation.

Keywords: Human bladder; β-adrenoceptor subtypes; functional analysis; mRNA analysis

Abbreviations: AMV, avian myeloblastosis virus; β-AR, β-adrenoceptor; DMSO, dimethyl sulphoxide; G3PDH, glyceraldehyde-3-phosphate dehydrogenase; RT-PCR, reverse transcription-polymerase chain reaction

Introduction

There is much evidence indicating that, at least in animals, activation of the sympathetic nervous system contributes to urine storage by relaxing the detrusor muscle *via* activation of β-adrenoceptors (β-ARs; see review by Andersson, 1993). Although β-ARs were originally subclassified into β_1- and β_2-subtypes (Lands *et al.*, 1967a,b), another subtype, the β_3-subtype, has since been reported (Emorine *et al.*, 1989; 1994). Furthermore, it has been known for some years that there are species differences in the subtypes of β-ARs involved in the relaxation of the detrusor (Elmér, 1974; Nergårdh *et al.*, 1977; Anderson & Marks, 1984; Levin *et al.*, 1988; Li *et al.*, 1992; Goepel *et al.*, 1997; Seguchi *et al.*, 1998). Recently, we reported that part of the relaxation induced by adrenergic stimulation in the rat and canine detrusor was mediated by β_3-AR (Yamazaki *et al.*, 1998).

In humans, although the β-ARs present in the detrusor muscle have been shown to have functional characteristics typical of neither β_1- nor β_2-ARs (Nergårdh *et al.*, 1977; Larsen, 1979), receptor-binding studies carried out using selective radio-ligands have indicated a predominance of the β_2-subtype (Levin *et al.*, 1988). It is still unclear whether β_3-ARs are present in the human detrusor and, if they are, what function they perform. In the present study, we set out to determine which β-AR subtypes are involved in the relaxation of the human detrusor that occurs on adrenergic stimulation. We were particularly interested in β_3-ARs, and we used *in vitro* functional studies and mRNA analysis. Some of the results have been presented in a preliminary communication (Igawa *et al.*, 1998).

Methods

Patients and specimens

The study involved 56 patients (44 men and 12 women; aged 66.2 ± 1.5, range 23–82 years) undergoing open pelvic surgery at Shinshu University Hospital, 38 for bladder carcinoma, eight for renal pelvic or ureteral carcinoma, two for prostatic carcinoma, three for bladder stone, three for benign prostatic hyperplasia, and two for vesico-ureteral reflex. On the basis of preoperative urodynamic studies and neurological examina-

*Author for correspondence; E-mail: yxigawa@gipac.shinshu-u.ac.jp

tions, all patients were judged to have normal bladder function. None of the patients had diseases known to interfere with the β-AR system or had received medication known to interfere with that system. General anaesthesia was induced with a short-acting barbiturate and was maintained with fentanyl and a mixture of oxygen, nitrous oxide and isoflurane. Written informed consent was obtained from all patients before their operation. The study was approved by the Ethics Committee of Shinshu University School of Medicine.

All specimens were taken from macroscopically normal tissue in the anterior or posterior wall of the bladder body *via* a longitudinal incision. In all cases, except when total cystectomy was performed for bladder carcinoma, specimens were obtained from the margin of the longitudinal incision in the anterior bladder wall during the operation itself. For functional studies, the specimens were placed immediately after excision in pre-oxygenated Krebs solution (for composition see below) at 4°C and transported to the laboratory. The specimens for RNA analysis were frozen immediately after excision and stored in liquid nitrogen.

Functional studies

After the mucosa and adventitia had been removed, detrusor muscle strips measuring approximately $10 \times 5 \times 3$ mm were isolated. Each preparation was suspended in a 10 ml organ bath containing Krebs solution; this was maintained at 37°C and continuously gassed with a mixture of 95% oxygen and 5% carbon dioxide. One end of each strip was connected to a force-displacement transducer (SB-1T, Nihon-Kohden, Tokyo, Japan) and changes in muscle tension were measured and recorded on a pen-writing oscillograph (Rectigraph 8S, NEC Sanei, Tokyo, Japan). The preparation was gradually stretched until a stable tension of 10 mN was obtained. Concentration-response curves for β-AR agonists were obtained by cumulative addition of the appropriate drug to the bathing fluid. Each preparation was used in only one experiment, i.e. to obtain one concentration-curve for one of the agonists. To test the antagonistic potency of β-AR antagonists against isoprenaline, one of the antagonists was added to the bath 30 min before the addition of isoprenaline. Concentration-response curves for isoprenaline were thus obtained in the presence of the antagonist. All experiments were conducted in the presence of 10^{-6} M phentolamine, an α-adrenoceptor antagonist.

Analysis of functional data

The results are expressed as means ± s.e.mean. The relaxing effect of each agonist is expressed as a percentage of the maximal relaxation induced by 10^{-5} M forskolin, which was used as a reference drug. The pD_2 value, which is the negative logarithm of the EC_{50} value, was calculated for each agonist from its concentration-relaxation curve. The pA_2 value for each antagonist, as defined by Arunlakshana & Schild (1959), was obtained from linear regression analysis of the plot of mean values of log (CR-1) vs the negative log of the antagonist concentration. When a parallel rightward shift of the concentration-relaxation curve was observed only at the highest concentration of antagonist used, the pK_B value (the negative logarithm of (antagonist concentration/CR-1)) was calculated from the pD_2 values in the presence (at the highest concentration) and absence of antagonist. Statistical analysis was performed using a Student's two-tailed *t*-test. A probability level of less than 0.05 was accepted as significant.

mRNA analysis

All procedures were carried out according to the manufacturer's instructions unless otherwise specified. Total RNA was extracted from human detrusor smooth muscle (approximate 1 g) using TRIzol® (Gibco-BRL, Rockville, MD, U.S.A.). The total RNA preparations were digested by RQ1 RNase-free DNase (Promega, Madison, WI, U.S.A.) to remove contaminating genomic DNA. The amount of the total RNA was determined by a spectrophotometer (DU-640, Beckman Instruments Inc., Fullerton, CA, U.S.A.).

Primers (Sawady Technology Inc., Tokyo, Japan) for β_1-, β_2-, and β_3-AR were used according to a previous report (Krief *et al.*, 1993) and primers for glyceraldehyde-3-phosphate dehydrogenase (G3PDH), as an internal standard, were designed in base on a DNA sequence (Tso *et al.*, 1985). The sequences of these primers are shown in Table 1. The reverse transcription-polymerase chain reaction (RT-PCR) was carried out using Titan™ one tube RT-PCR system (Boehringer-Mannheim, Mannheim, Germany). The PCR mixtures consisted of 1 × the RT-PCR buffer (containing $MgCl_2$ (1.5 mM) and dimethyl sulphoxide (DMSO); Boehringer-Mannheim), dNTPs (0.2 mM), 0.4 μM each of PCR primers, DTT (5 mM), 5 U RNasin® RNase inhibitor (Promega, U.S.A.), 1 μl enzyme mix (expand™ high fidelity enzyme mix and avian myeloblastosis virus (AMV)-reverse transcriptase) and 1 μg total RNA, in a volume of 50 μl. cDNA synthesis and PCR amplification was continuously performed without opening the reaction tubes in a thermal cycler (Gene Amp® 2400, PE Applied-Biosystems, Foster City, CA, U.S.A.).

cDNA synthesis was performed at 50°C for 30 min with reverse transcriptase and PCR primer. Following initial heating of samples at 94°C for 2 min, 10 cycles were performed, which consisted of denaturation (30 s at 94°C), annealing (30 s at 58°C) and elongation (1 min at 68°C).

Table 1 Oligonucleotides used as RT-PCR primers

	Strand	*Sequence*	*Location**
β_1-adrenoceptor	Forward	5′-TCGTGTGCACCGTGTGGGCC-3′	J03019
	Reverse	5′-AGGAAACGGCGCTCGCAGCTGTCG-3′	(619–883)
β_2-adrenoceptor	Forward	5′-GCCTGCTGACCAAGAATAAGGCC-3′	Y00106
	Reverse	5′-CCCATCCTGCTCCACCT-3′	(1221–1549)
β_3-adrenoceptor	Forward	5′-GCTCCGTGGCCTCACGAGAA-3′	X70811
	Reverse	5′-CCCAACGGCCAGTGGCCAGTCAGCG-3′	(129–442)
G3PDH	Forward	5′-ACCACAGTCCATGCCATCAC-3′	X01677
	Reverse	5′-TCCACCACCCTGTTGCTGTA-3′	(586–1037)

*Genbank accession number and nucleotide numbers within corresponding entry.

Furthermore, the PCR amplification was repeated for 25 cycles for β_1-, β_2- and β_3-AR and for 14 cycles for G3PDH in the same manner except that the elongation time was added 5 s for each cycle (These numbers of repeated cycles and the elongation times were determined by results of pilot experiments).

A portion (10 μl) of PCR-products were visualized by electrophoresis of 3% LO3 agarose gels (TAKARA-Shuzo, Ohtsu, Japan) with 0.5 μg ml^{-1} ethidium bromide (Gibco-BRL). To identify these PCR-products, direct sequencing of PCR-products was performed according to the dideoxy chain termination method. The PCR-products were labelled by Big-dye terminator (PE Applied-Biosystems) using the sense and anti-sense PCR primers, and analysed by DNA sequencer (ABI PRISM™ 310, PE Applied-Biosystems).

Drugs and solutions

The following drugs were used; (±)-isoprenaline hydrochloride, forskolin (Wako Pure Chemical, Osaka, Japan), (±)-dobutamine hydrochloride, BRL37344A ((±)-(R*,R*)-[4-[2-[[2-(3-chlorophenyl)-2-hydroxyethyl]-amino]propyl]phenoxy]-acetic acid sodium), (±)-CGP-12177A hydrochloride ((±)-4-(3-t-butylamino-2-hydroxypropoxy) benzimidazol-2-one hydrochloride), ICI-118,551 hydrochloride (erythro-(±)-1-(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol hydrochloride) (Funakoshi, Tokyo, Japan), procaterol hydrochloride (Sigma Chemical, St. Louis, MO, U.S.A.), (±)-noradrenaline (Sankyo, Tokyo, Japan), (−)-adrenaline (Daiichi, Tokyo, Japan), phentolamine mesylate (Ciba-Geigy, Basel, Switzerland) and dimethyl sulphoxide (DMSO) (Nacalai tesque, Kyoto, Japan). CL316243((R,R)-5-[2-[[2-(3-chlorophenyl)-2-hydroxyethyl]-amino]propyl]- 1,3-benzodioxole-2,2-dicarboxylate), CGP-20712A (2-hydroxy-5(2-((2-hydroxy-3-(4-((1-methyl-4-trifluoromethyl)1H-imidazole-2-yl)-phenoxy)propyl) amino) ethoxy)-benzamide monomethane sulphonate) and SR58894A (3-(2-allylphenoxy)-1-[(1S)-1,2,3,4-tetrahydronaphth-1-ylamino]-(2 S)-2-propanol hydrochloride) were synthesized in our laboratories (Kissei, Hotaka, Japan). The drugs were dissolved as follows: forskolin, in 100% DMSO; the other drugs, in distilled water. The solutions were prepared on the day of the experiment and kept in dark vessels to minimize light-induced degradation. Subsequent dilutions of the drugs were prepared in distilled water. The reported concentrations are the calculated final concentrations in the bath solution. The Krebs solution used had the following composition (mM): NaCl 118.1, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25.0, KH_2PO_4, 1.2 and glucose 11.1 (pH 7.4).

Results

Relaxation responses to β-AR agonists

A distinct relaxation of the human detrusor preparation was produced by forskolin (10^{-5} M), the tension decreasing to 47.3±2.4% (n=60) of the initial tension. Isoprenaline (10^{-10}–10^{-4} M), a non-selective β-AR agonist, produced relaxation in a concentration-dependent manner (Figure 1). The maximal effect, which was observed at a concentration of 10^{-4} M, averaged 78.7±1.4% (n=29) of the forskolin (10^{-5} M)-induced maximal relaxation. Both noradrenaline (10^{-10}–10^{-4} M) and adrenaline (10^{-10}–10^{-4} M) also relaxed the preparations in a concentration- dependent manner. The rank order for the relaxing activity of these drugs was isoprenaline ⩾ noradrenaline ⩾ adrenaline, the pD_2 values being 6.37±0.07 (n=29), 6.07±0.12 (n=6) and 5.88±0.11 (n=6), respectively (Figure 2). The difference in pD_2 value between isoprenaline and adrenaline was statistically significant (P<0.05). However, there was no statistically significant difference in pD_2 value between isoprenaline and noradrenaline, or between noradrenaline and adrenaline.

On the other hand, neither dobutamine (10^{-10}–10^{-4} M), which stimulates both β_1- and β_2-ARs, nor procaterol (10^{-10}–10^{-4} M), a selective β_2-AR agonist, produced any significant relaxation at concentrations up to 10^{-5} M (Figure 2). When

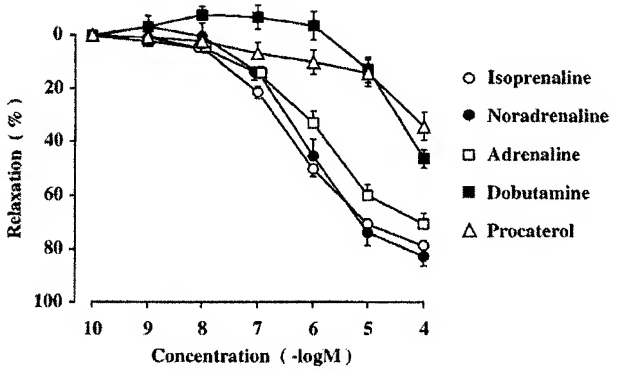

Figure 2 Effects of isoprenaline, noradrenaline, adrenaline, dobutamine, and procaterol on resting tension in human detrusor preparations. All experiments were performed in the presence of 10^{-6} M phentolamine. Each point represents the mean±s.e.mean of 6–29 experiments. Data are expressed as a percentage of the maximal relaxation induced by 10^{-5} M forskolin.

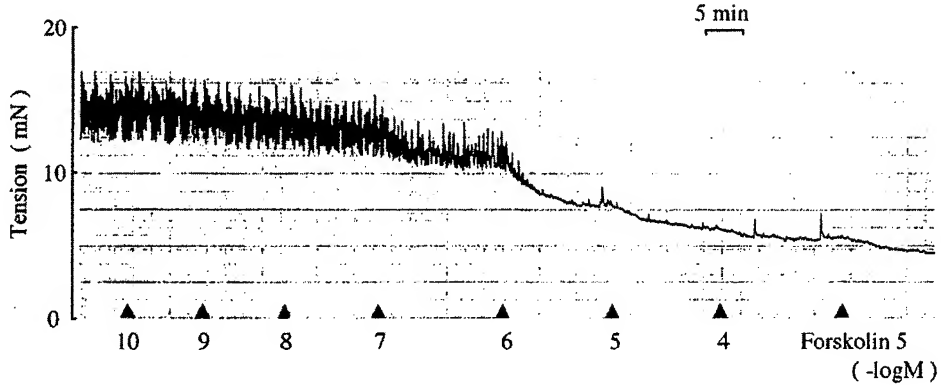

Figure 1 Representative recording of the effect of isoprenaline on resting tension in a human detrusor preparation.

applied at 10^{-4} M, dobutamine and procaterol produced relaxing effects that were the equivalent of $46.2\pm3.4\%$ ($n=8$) and $34.2\pm5.2\%$ ($n=11$), respectively, of the forskolin (10^{-5} M)-induced relaxation. However, neither of these effects reached a maximum at a concentration of 10^{-4} M and so the pD_2 values were not determined.

Both BRL37344A ($10^{-10}-10^{-4}$ M) and CL316243 ($10^{-10}-10^{-4}$ M), selective β_3-AR agonists, and CGP-12177A ($10^{-10}-10^{-4}$ M), a selective β_3-AR partial agonist and β_1-/β_2-AR antagonist, relaxed the preparation when applied at concentrations greater than 10^{-6} M (Figure 3). The pD_2 values (and maximal relaxation at 10^{-4} M) were 6.42 ± 0.25 ($47.4\pm4.5\%$; $n=7$) for BRL37344A, 5.53 ± 0.09 ($43.7\pm6.4\%$; $n=7$) for CL316243 and 5.74 ± 0.14 ($33.3\pm4.2\%$; $n=11$) for CGP-12177A, respectively.

Effect of β-AR antagonists on the isoprenaline-induced relaxation

CGP-20712A ($10^{-7}-10^{-5}$ M; $n=5-6$), a selective β_1-AR antagonist, failed to affect the concentration-relaxation curve for isoprenaline (Figure 4a). At concentrations from 10^{-7} M to 3×10^{-6} M, ICI-118,551 ($n=6-7$), a selective β_2-AR antagonist, did not affect the relaxation induced by isoprenaline. However, at 10^{-5} M ICI-118,551 produced a rightward parallel shift of the isoprenaline concentration-response curve (Figure 4b). The pK_B value determined by using 10^{-5} M ICI-118,551 was 5.71 ± 0.19.

In the presence of both CGP-20712A (10^{-7} M) and ICI-118,551 (10^{-7} M), on the other hand, addition of SR58894A ($10^{-7}-10^{-5}$ M; $n=7-12$), a selective β_3-AR antagonist, caused a rightward shift of the concentration-relaxation curve for isoprenaline in a concentration-dependent manner (Figure 5a). A Schild plot analysis yielded a pA_2 value of 6.24 ± 0.20 and a slope of 0.68 ± 0.31 (Figure 5b). The slope did not differ from unity for this antagonist.

Expression of β-AR mRNA in human detrusor

RT-PCR amplification was carried out using specific primers corresponding to β_1-, β_2- and β_3-AR sequences and, as a template, mRNA obtained from human detrusor preparations from three patients. As shown in Figure 6, PCR products for β_1-, β_2- and β_3-AR were detected identically in all the preparations and the expected size of PCR products for β_1-, β_2- and β_3-AR were 265, 329 and 314 bp, respectively. The PCR product for G3PDH, an internal standard, was detected also in each preparations obtained from all the three patients and its expected size was 452. The sequences of the PCR products analysed by DNA sequencer were identified with their reported sequences as regards the detectable signals.

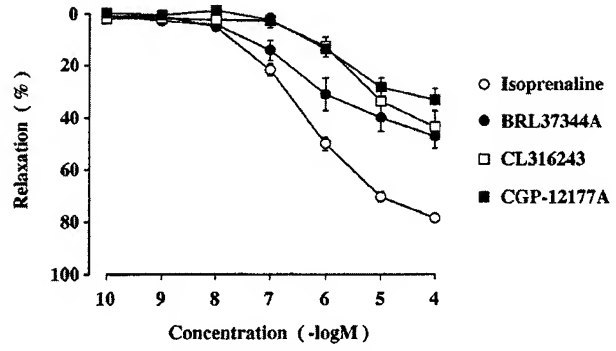

Figure 3 Effects of isoprenaline, BRL37344A, CL316243 and CGP-12177A on resting tension in human detrusor preparations. All experiments were performed in the presence of 10^{-6} M phentolamine. Each point represents the mean±s.e.mean of 7–29 experiments. Data are expressed as a percentage of the maximal relaxation induced by 10^{-5} M forskolin.

Discussion

The present study, combining functional and molecular biological investigations, provides evidence for the existence of β_3-AR in the human detrusor. It further suggests that the major β-AR subtype involved in the relaxation of the human detrusor smooth muscle observed on adrenergic stimulation is neither the β_1- nor the β_2-AR, but most probably the β_3-AR.

First, we examined the relative potencies with which endogenous and synthetic catecholamines relaxed the human detrusor muscle. The rank order of potency for the three catecholamines producing β-AR-mediated responses has been reported to be isoprenaline > noradrenaline > adrenaline for β_1- and β_3-ARs, but isoprenaline > adrenaline > noradrenaline for β_2-AR (Lands *et al.*, 1967a; Emorine *et al.*, 1989). In the present study, the rank order of potency in relaxing human detrusor was isoprenaline ≥ noradrenaline ≥ adrenaline. Although the pD_2 value for noradrenaline was greater than that for adrenaline, the difference was not statistically

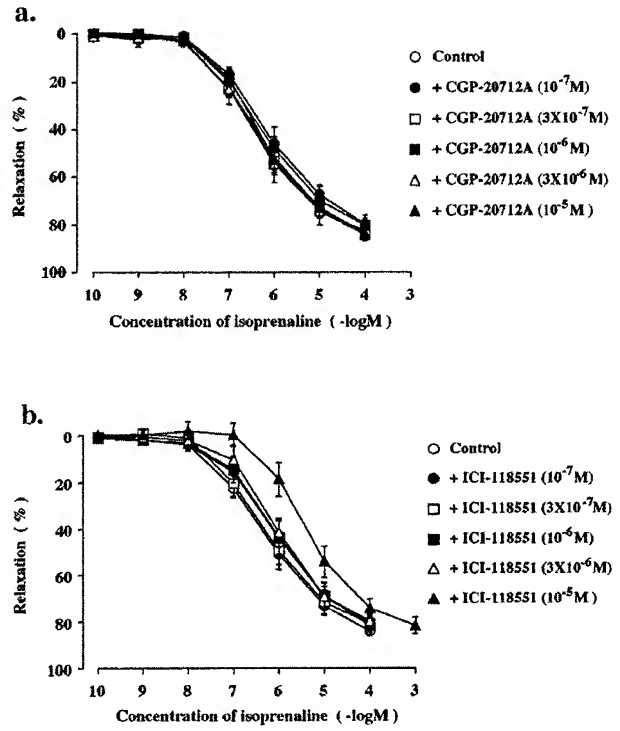

Figure 4 Effects of CGP-20712A (a) and ICI-118,551 (b) on the isoprenaline-induced relaxation of human detrusor muscle preparations. (a) Concentration-response relationships for isoprenaline, either alone or in the presence of CGP-20712A 10^{-7} M, 3×10^{-7} M, 10^{-6} M, 3×10^{-6} M or 10^{-5} M. Each point represents the mean±s.e.mean ($n=5-6$). (b) Concentration-response relationships for isoprenaline, either alone or in the presence of ICI-118,551 10^{-7} M, 3×10^{-7} M, 10^{-6} M, 3×10^{-6} M, or 10^{-5} M. Each point represents the mean±s.e.mean ($n=6-7$). All experiments were carried out in the presence of 10^{-6} M phentolamine. Data are expressed as a percentage of the maximal relaxation induced by 10^{-5} M forskolin.

significant. Thus, no difinitive conclusions in terms of a functional predominance of either subtype of β-AR in the human detrusor can be drawn.

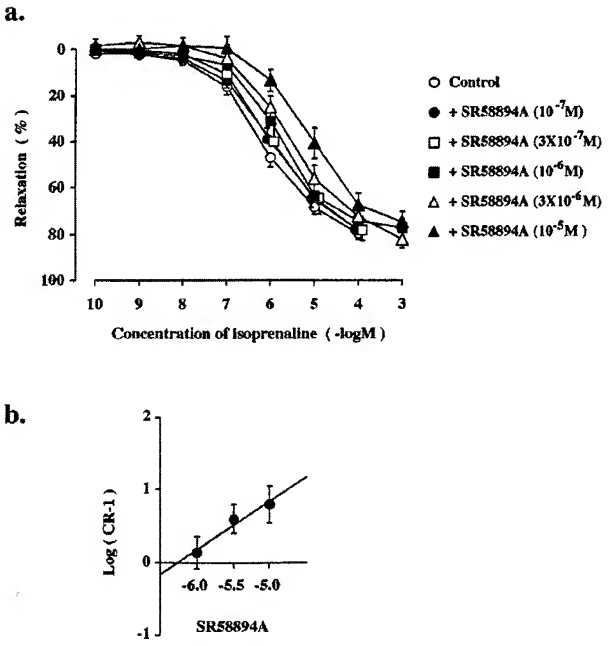

Figure 5 Effect of SR58894A on the isoprenaline-induced relaxation of human detrusor muscle preparations. All experiments were carried out in the presence of CGP-20712A (10^{-7} M), ICI-118,551 (10^{-7} M) and phentolamine (10^{-6} M). (a) Concentration-response relationships for isoprenaline, either alone or in the presence of SR58894A 10^{-7} M, 3×10^{-7} M, 10^{-6} M, 3×10^{-6} M or 10^{-5} M. Each point represents the mean±s.e.mean ($n=7-12$). Data are expressed as a percentage of the maximal relaxation induced by 10^{-5} M forskolin. (b) Schild plot for the inhibition of the isoprenaline-induced relaxation produced by SR58894A.

Second, we tested the potencies with which selective agonists for the β-AR subtypes relaxed the human detrusor muscle. Neither dobutamine, which stimulates both β_1- and β_2-ARs (Ozaki *et al.*, 1982; Ruffolo *et al.*, 1984; Ruffolo, 1987; Aikawa *et al.*, 1996), nor procaterol, a β_2-AR agonist, produced any significant relaxation at up to 10^{-5} M. In fact, the concentration at which dobutamine and procaterol did induce a relaxing effect in our preparation was around 10^{-4} M. At this concentration, it is doubtful that the drugs stimulated any β-AR subtype selectively.

We then examined the relaxing effects of the selective β_3-AR agonists, BRL37344A (Oriowo *et al.*, 1996), CL316243 (Bloom *et al.*, 1992) and CGP-12177A. CGP-12177A has been reported to be a partial agonist for the β_3-AR and an antagonist for β_1- and β_2-ARs (Kaumann, 1996). These β_3-AR agonists were more potent in relaxing our preparations than either dobutamine or procaterol. Thus, these findings suggest that the β_3-AR is functionally predominant over β_1- and β_2-ARs in the human detrusor. Although the β_3-AR agonists, BRL37344A and CL316243, produced relaxations in the human detrusor at concentrations over 10^{-6} M, the maximal relaxations induced by these β_3-AR agonists were only half of the maximal relaxation induced by isoprenaline. This suggests that other β-ARs than the β_3-AR may coexist and contribute to the relaxation of the human detrusor.

Confirmation of the predominant role of the β_3-AR in the human detrusor was obtained in our investigation of the potencies with which several β-AR antagonists counteracted the isoprenaline-induced relaxation of our preparations. In fact, CGP-20712A, a selective β_1-AR antagonist, did not affect the isoprenaline-induced relaxation. Moreover, ICI-118,551, a selective β_2-AR antagonist, shifted the concentration-relaxation curve for isoprenaline only at a high concentration (10^{-5} M). But, the shift was parallel and its pK_B value (5.71) was comparable to the pA_2 value of 5.31 for the antagonist in antagonizing isoprenaline-induced relaxation of rat oesophageal muscularis mucosae, which is known to be mediated predominantly through β_3-ARs (De Boer *et al.*, 1993) and the

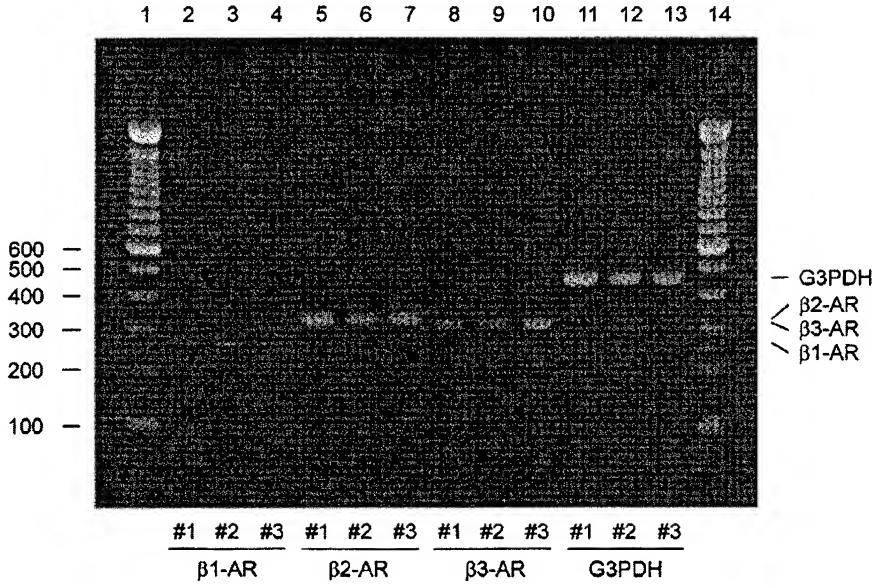

Figure 6 Detection of β_1-, β_2- and β_3-AR mRNA in human detrusor tissue by RT-PCR. Detrusor preparations were obtained from three different patients (#1, #2 and #3). PCR amplification for β_1- (lanes 2–4), β_2- (lanes 5–7), β_3-AR (lanes 8–10) and G3PDH (lanes 11–13) were carried out for 35, 35, 35 and 24 cycles, respectively. Expected size of PCR-products for β_1-, β_2-, β_3- AR and G3PDH were 265, 329, 314 and 452, respectively. Lanes 1 and 14 show 100 bp DNA ladder (Gibco-BRL).

pK_B value of <5.5 for rat cardiac putative β_4-AR (Kaumann, 1997). This suggests that the functional β-ARs in the human detrusor belong to neither the β_1- nor β_2-AR subtypes, but probably to some other subtype (β_3- or β_4).

We then studied the antagonistic activity of SR58894A, a recently developed β_3-AR selective antagonist (Manara *et al.*, 1996), against the isoprenaline-induced relaxation. In the presence of 10^{-7} M CGP-20712A (β_1-AR antagonist) and 10^{-7} M ICI-118,551 (β_2-AR antagonist), SR58894A counteracted the isoprenaline-induced relaxation of the human detrusor. This finding provides functional evidence for β_3-ARs in the human detrusor. It has been reported that bupranolol, a non-selective β-AR antagonist, has an antagonistic action at β_1- and β_2-ARs at low concentrations (nM), whereas at higher concentrations (μM) it also affects the β_3-AR (Kaumann, 1989; Koike *et al.*, 1995). In a preliminary study (Igawa *et al.*, 1998), pretreatment with bupranolol at 10^{-7}, 10^{-6} and 10^{-5} M produced an apparent rightward shift of the concentration-response curve for isoprenaline. Its pA_2 value for this effect was 7.27, which is comparable to the pA_2 values (about 7.3–7.5) for β_3-AR reported by Langin *et al.* (1991). Thus, the findings with bupranolol further support the view that the relaxation induced by adrenergic stimulation in the human detrusor is mediated mainly by β_3-ARs, rather than by β_1- or β_2-ARs. However, the slope for SR58894A, obtained from Schild plots, was 0.68. This suggests that other β-ARs, possibly the putative β_4-AR (Kaumann, 1997) and/or atypical β-ARs, may coexist and play a functional role in the relaxation of the human detrusor. Indeed, the presence of atypical β-ARs has been postulated in human colonic smooth muscle (De Ponti *et al.*, 1996). Further studies are needed to determine whether such ARs exist also in the human detrusor.

To obtain a biological correlate of the pharmacological evidence for a functional β_3-AR in the human detrusor, we used a PCR assay to study the expression of the mRNA for each of the various β-AR genes. In fact, the mRNAs for all three β-AR subtypes were demonstrated in the human detrusor in the present study. The DNA sequencer analysis performed subsequently confirmed that each sequence of these PCR products was identified with its reported sequence. Although the distribution of the β_3-AR mRNA has been investigated in a variety of human tissues, such as white fat, gall bladder, small intestine, stomach and prostate (Krief *et al.*, 1993; Berkowitz *et al.*, 1995), to our knowledge, this is the first report of a distribution of β_3-AR mRNA in the human urinary bladder. A putative β_4-AR has been reported in the human heart (Kaumann, 1997). If β_4-AR cDNA is isolated and its DNA sequence is determined, more detailed analysis of the expression of β-AR would be possible.

Taken together, the results of the present pharmacological study and those of the mRNA analysis indicate the presence of the β_3-AR in the human detrusor. They further suggest that the relaxation of the human detrusor produced by adrenergic stimulation is mediated mainly by the β_3-AR.

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